Developmental Cell
Previews

First Time, Every Time: Nucleosomes at a Promoter Can Determine the Probability of Gene Activation

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DOI 10.1016/j.devcel.2010.04.006

Transcription factor binding sites are found in either nucleosome-free or nucleosome-embedded locations, thus in vivo relationships between nucleosome position and gene activation are not fully understood. In this issue of *Developmental Cell*, Bai et al. show that binding sites located in nucleosome depleted regions guarantee high reliability, not amplitude, of promoter firing.

A nucleosome can block transcription factor binding, and global surveys show most promoters have upstream nucleosome depleted regions (NDRs), while others have nucleosomes that may block transcription factor binding. What are the effects of inhibitory nucleosomes on gene activation? In this issue of *Developmental Cell*, Bai et al. (2010) examine this question at a cell-cycle regulated promoter and show that a promoter NDR can guarantee that a gene is activated every cycle. Alternatively, nucleosome-occupied binding sites can create a bimodal pattern of activation that may require stricter conditions for activation.

The *S. cerevisiae CLN2* gene encodes a G1 cyclin, and cyclin expression drives the G1/S transition (Skotheim et al., 2008). Bai et al. (2010) mapped positions of nucleosomes at the *CLN2* promoter and found a nucleosome covering the TATA element and the transcription start site (Figure 1A). The NDR between nucleosomes −2 and −3 contains three binding sites for SBF, a G1-specific transcription factor. SBF is inactive in early G1, but phosphorylation of the Whi5 inhibitor by CDK1 ends this inhibition, allowing SBF to activate *CLN2* (Costanzo et al., 2004; de Bruin et al., 2004). Studies with synchronized cells show that nucleosomes −1 and −2 are evicted transiently during the cell-cycle and that nucleosome eviction requires SBF and the FACT histone chaperone (Bai et al., 2010; Takahata et al., 2009a).

While many groups investigate transcription in bulk experiments, measuring factor binding or mRNA levels in a population of cells, the Cross laboratory has examined activation of the *S. cerevisiae CLN2* G1 cyclin gene in single-cell fluorescence assays (e.g., Skotheim et al., 2008). They used a destabilized GFP reporter under the control of the *CLN2* promoter to determine when *CLN2* is expressed, and Myo1-mCherry, which marks the bud neck but disappears at cytokinesis, allowing one to recognize exactly when a cell is born. Measuring the time between cell birth and *CLN2pr::GFP* appearance in individual cells previously allowed demonstration of a positive-feedback loop for G1 cyclins, something that had been obscured in bulk population studies (Skotheim et al., 2008).

CLN2pr::GFP was expressed reliably every cell cycle, while deleting the SBF sites eliminated expression. Starting with this *CLN2pr::GFP* promoter lacking SBF sites, the authors reintroduced SBF sites either into an NDR (*SBF in NDR*) or embedded the SBF sites within a nucleosome (*SBF in Nuc*) (Figure 1B). The *SBF in NDR* construct functioned like the native promoter, but the *SBF in Nuc* promoter was quite different. While one might have expected reduced expression from *CLN2pr::GFP* with nucleosomal SBF sites, surprisingly, it was expressed at the same level, but in only 75% of cell cycles. Thus, placing the SBF factor binding sites within a nucleosome resulted in either "on" or "off" states in individual cell cycles, rather than simply reduced expression. This suggests that only sometimes is the factor able to bind and promote nucleosome eviction, while in other cell cycles transcription does not occur; the authors call this "bimodal expression." Remarkably, cells with this *SBF in Nuc* promoter exhibited a short-term memory, with an "on" cell more likely to produce "on" cell progeny, and "off" cells more likely to produce "off" cells.

To further characterize the relationship of SBF and nucleosomes, the authors examined *HO*, another SBF-dependent gene. The SBF binding sites at *HO* are embedded within nucleosomes, and these nucleosomes are evicted transiently during the cell cycle (Bai et al., 2010; Takahata et al., 2009b). The SBF sites at *HO* alone are insufficient to activate expression, and coactivators recruited to an upstream promoter region are required for expression and for eviction of the SBF nucleosomes (Cosma et al., 1999; Takahata et al., 2009b). Bai et al. (2010) made several chimeric promoters from *CLN2* and *HO*, driving the unstable GFP reporter. Constructs with only a small region of *HO* inserted into *CLN2* resulted in the *HO* SBF sites being in an NDR, and the gene displayed unimodal expression in every cell. In contrast, nucleosome positioning was retained on the SBF sites when a larger *HO* DNA fragment was inserted into *CLN2*. This hybrid promoter exhibited mostly the "off" condition, with expression in only 8% of cells. Additionally, this promoter displayed short-term memory, like the *SBF in Nuc* promoter. Thus, SBF sites present in an NDR again produced a promoter that is reliably activated every cell cycle, while nucleosome-embedded SBF sites result in highly stochastic promoter firing only in a subset of cell cycles.

Bai et al. (2010) clearly show that a transcription factor binding site in an NDR can result in very efficient gene activation, while the same binding site embedded within a nucleosome can result in activation in only some cell cycles, with this promoter displaying short-term memory or epigenetic inheritance. This work raises important mechanistic questions. What

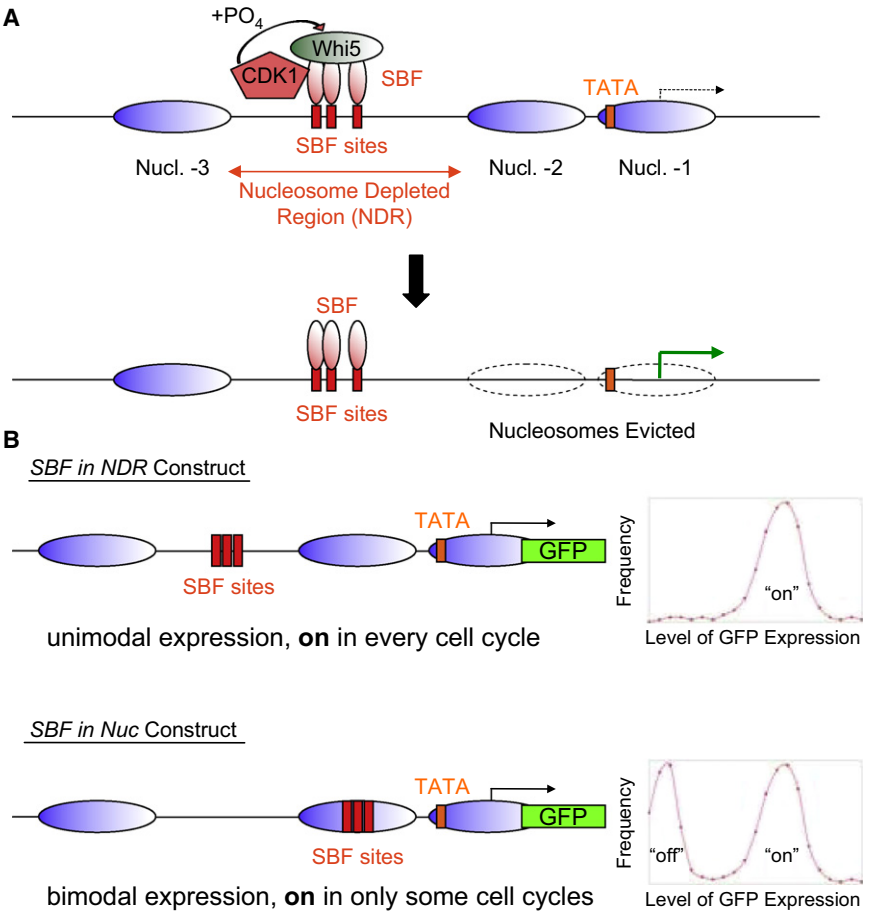

Figure 1. An SBF Site in a Nucleosome-Free Region (NDR) Results in *CLN2* Expression Every Cell Cycle

(A) The arrangement of nucleosomes and SBF binding sites in the *CLN2* promoter. Once CDK1 phosphorylation removes the Whi5 inhibitor, nucleosomes −1 and −2 are evicted and *CLN2* is transcribed. Nucleosomes are reassembled after *CLN2* expression subsides.

(B) Single-cell reporters with internucleosome versus nucleosome-embedded SBF sites. NDR-located SBF sites program the expression of GFP at a high frequency, with a high probability of expression in each cell and in each cell cycle. Conversely, nucleosome-embedded SBF sites allow GFP expression more rarely, with a high frequency of cells with no expression, as well as some cells in which the promoter still fires at normal levels of expression.

determines whether a DNA segment is nucleosomal or nucleosome-free? The SBF sites in a 550 bp region of *HO* were nucleosomal when transplanted into *CLN2*, but three smaller segments spanning this *HO* region were nucleosome-free when moved into *CLN2*. What factors are required for nucleosomal eviction at the bimodal promoters? Bai et al. (2010) present evidence that that the Swi/Snf remodeler increases the frequency that a bimodal promoter can be activated, as do mutations eliminating a histone deacetylase. This latter observation is consistent with recent reports showing *CLN2* regulation by histone deacetylases (Huang et al., 2009; Takahata et al., 2009a; Wang et al., 2009). Finally, what is the stochastic process that leads to activation of bimodal promoters? Overexpression of one of the subunits of SBF increased the probability that a bimodal promoter would be expressed, suggesting that SBF binds inefficiently to sites within nucleosomes. *CLN2* is a gene that should be expressed each and every cell cycle, and its transcription factor binding sites are in NDRs. In contrast, inducible and developmentally regulated promoters, with pioneer transcription factor binding sites positioned naturally within nucleosomes (Sekiya et al., 2009), can limit expression of genes important for developmental decisions. The work of Bai et al. (2010) provides us with a mechanistic underpinning for how different promoters have different probabilities for activation.

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